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PRINCIPAL INVESTIGATOR: Stefan M. Pulst, M.D.

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center
Los Angeles, CA 90048

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Stefan M. Pulst, M.D.

Email: pulst@cshs.org

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Neurofibromatosis 2 (NF2) is a tumor suppressor gene syndrome characterized by the development of tumors of Schwann cell, meningeal, and ependymal origin. NF2 is also the gene most commonly mutated in sporadic tumors of these cell types. With previous support from the NF2 program of the Army Medical Branch we have identified hepatocyte growth factor regulated kinase substrate (HRS) as a protein that interacts with schwannomin. Studies by us and others have indicated a role for HRS in growth factor receptor trafficking and downregulation of signaling, but also established a role for HRS in cytokine and IGF1-mediated signaling to the STAT pathway. In the first year of funding, we have continued our *in vitro* studies of Hrs partial proteins and have initiated mouse studies to test interactions. We have identified several different Hrs molecules that show dominant negative effects. Our initial intercrosses of *Hrs*$^{+/-}$ mice with *Nf2*$^{+/-}$ mice have been successful. However, we have noticed slightly reduced litter size and some evidence for segregation distortion. This may influence our overall timetable in that it may be more costly and lengthy to produce the number of animals necessary for analysis.

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## Introduction:

Although autosomal dominant NF2 is relatively rare with an incidence of 1 in 40,000, studies by us and others have demonstrated that virtually all sporadic schwannomas and 50% of ependymomas and meningiomas harbor NF2 mutations. NF2 mutations have been identified in tumors and tumor cell lines in a variety of other human tumors. These include breast and lung cancer, melanoma, and mesothelioma. Especially in mesotheliomas, highly malignant asbestos-induced cancers of the pleura, NF2 mutations have repeatedly been found to contain NF2 mutations and loss of NF2 protein.

The investigation of NF2 function has been greatly aided by the identification of NF2-interacting proteins and the development of animal models. This proposal has for the first time combined these two approaches. We have identified HRS as an interactor of the NF2 protein. HRS and schwannomin interact physically and functionally.

When we identified HRS as an NF2 interactor, relatively little was known about this protein. It had been identified as a protein that was phosphorylated in response to treatment of cells with Hepatocyte Growth Factor (HGF, also called scatter factor). It is a 775 amino acid protein with a predicted MW of 110 kDa. Using antibody-labeling and subcellular fractionation it was shown that HRS had an almost ubiquitous expression pattern and was localized to the outside of endosomes. Furthermore, homology to the yeast protein Vps27p, which is essential for protein traffic through a pre-vacuolar compartment in yeast, suggested a role for HRS in vesicular transport through early endosomes. Studies by us and others have indicated a role for HRS in ERB receptor trafficking and downregulation of EGFR signaling, but also in cytokine and IGF1-mediated signaling to the STAT pathway. HRS has been shown to be important in signaling in pathways that are relevant to Schwann cell differentiation and proliferation.

Although great strides have recently been made to re-introduce *NF2* into human NF2 schwannoma cells *in vitro*, results between the two groups that pioneered these approaches are only partially overlapping (morphologic changes vs. apoptosis). The difficulties in generating a schwannoma phenotype in *vitro* may be in part related to the fact that Schwann cell proliferation and differentiation are regulated by various signals including axonal electrical activity, adhesion to axons, basal lamina, and adjacent Schwann cells. In addition, phosphorylation status, isoform expression and subcellular localization of schwannomin vary depending on conditions and cellular context *in vitro*; the relevance of these variables is not known *in vivo*.

Animal models for NF2 have been very successful with two major caveats. *Nf2*$^{+/-}$ mice do develop tumors, but do not develop the tumor types typical for human NF2 patients, i.e. schwannoma, meningiomas, or ependymomas. When NF2-deficiency is targeted to Schwann cells, schwannomas develop, but usually relatively late.

By crossing *Nf2*$^{+/-}$ mice with *Hrs*$^{+/-}$ mice we hope to produce a tumor spectrum that includes the development of schwannomas. We will also target HRS-deficiency to Schwann cells using expression of dominant-negative *HRS* alleles. We expect to generate an animal model that will develop schwannomas more reliably and potentially earlier than currently available models.

# Body

## Task 1: *In vitro* interactions of NF2 and HRS

## Statement of work year 3

### Task 1:

No experiments to be completed in SoW year 3.

### Task 2: Effects of Hrs haploinsufficiency on *NF2*$^{+/-}$ mice

#### *Year 1: Hrs*$^{+/-}$ *intercross with Nf2*$^{+/-}$

*We will perform an intercross of Hrs*$^{+/-}$ *mice with Nf2*$^{+/-}$ *(mice Nf2*$^{+/KO3}$*). As there is no evidence for segregation distortion for these alleles in previous publications, we expect to obtain the following genotypes:*
*25% Hrs*$^{+/+}$*;Nf2*$^{+/+}$ *, 25% Hrs*$^{+/+}$*;Nf2*$^{+/-}$*, 25% Hrs*$^{+/-}$*;Nf2*$^{+/+}$*, 25% Hrs*$^{+/-}$*;Nf2*$^{+/-}$*.*

#### *Years 2 and 3 phenotypic analyses*

We had reported initial phenotypic results of 29 animals at the end of year 2. As we had proposed to follow 20 animals in each group of the F1 genotypes we have continued to breed animals and generate mice with the expected genotypes in a mixed FVB/N and 50%C57BL/6J background.

We have now accomplished this task and have completed a phenotypic and complete pathologic analysis of 125 mice. The following numbers were studied for each genotype:

Nf2$^{+/+}$ */ Hrs*$^{+/+}$ *: 41*
Nf2$^{+/-}$ */ Hrs*$^{+/+}$ *: 22*
Nf2$^{+/+}$ */ Hrs*$^{+/-}$ *: 33*
Nf2$^{+/-}$ */ Hrs*$^{+/-}$ *: 29*

An additional 20 mice were sacrificed at the end of January prior to the move of the laboratory to the University of Utah. The necropsy results for these mice will be included in the next progress report.

We hade previously concluded (based on much smaller numbers) that all genotypes are viable and that there is a subtle segregation distortion.

After 2.5 years we can now confirm this observation. All genotypes are indeed viable. We have continued to observe some segregation distortion with Nf2 haploinsufficient (Nf2$^{+/-}$*)* animals born at a reduced number. This, however, did not reach statistical significance (chi-square = 6.04, degrees of freedom: 3; p=0.1).

### Survival analysis:

The reported survival curves for wildtype and Nf2$^{+/-}$ mice begin to diverge at about 1 year (McClatchey et al., 1998). No formal survival data for *Hrs*$^{+/-}$ mice are known.

We now have a sufficient number of animals to conclude that there is no difference in survival for the four genotypes. Of the 138 animals, only 13 died during the observation period of 18 months. There were 8 deaths prior to 18 months for double heterozygotes, 2 each for single heterozygotes and 1 for wildtype. None of the animals had any visible tumors.

### Tumor analysis

We have continued our detailed necropsy analysis of the various mouse lines (wildtype, single heterozygotes and double heterozygotes). As very few animals showed any visible signs of tumor

development or other signs of illness, we decided to sacrifice animals as soon as they reach 18 months of age.

The animals are perfused with formalin and then undergo a formal necropsy with the aid of Dr. Serguei Bannykh, a pathologist with the subspecialty of neuropathology. As noted in the prior progress report, Dr. Bannykh has replaced Dr. Yong in this application, but has identical qualifications as a board-certified pathologist and neuropathologist. The results of the pathological examination are shown in two tables at the end of the progress report.

A complete survey was taken from the following organs: heart, lung, liver, kidney, pancreas, testis, epididymis, ovary, uterus, stomach, small and large bowel, salivary, lacrimal and adrenal glands, prostate and seminal vesicles, lymph nodes, bone marrow, lens of the eye, brain, meninges and spinal cord. Of note, the necropsy analysis was carried out with Dr. Bannykh blinded to the genotype of the respective animal.

### *Abnormalities and Tumors in the nervous system and eye (Table 1)*

At 18 months, we detected a number of abnormalities including basal ganglia calcifications, inflammatory infiltrates, and mild axonal degeneration. These abnormalities, however, occurred in all genotypes. Only one malignant peripheral nerve sheath tumor (MPNST) was seen in 125 necropsies. Contrary to our primary hypothesis, we have so far not observed an enhancement in the development of nervous system tumors.

### *Abnormalities and Tumors in other organs (table 2)*

Table 2 highlights the importance of our decision to increase the numbers of animals studied. It was our previous hypothesis based on a smaller number of mice (especially a small number of wildtype mice) that deficiency of NF2 combined with Hrs deficiency leads to the formation of adenocarcinomas of the lung as these tumors were only observed in double heterozygotes. Double heterozygotes also showed hepatocellular carcinoma or nuclear hyperplasia, again abnormalities that were not identified in any of the other mouse lines except for one Nf2+/- mouse.

We now conclude that these initial observations cannot be confirmed. After analyses of an adequately powered sample, there was no indication that the tumor spectrum in the three mutant genotypes deviated from that seen in wildtype mice in a mixed FVB/N:C57BL/6J background.

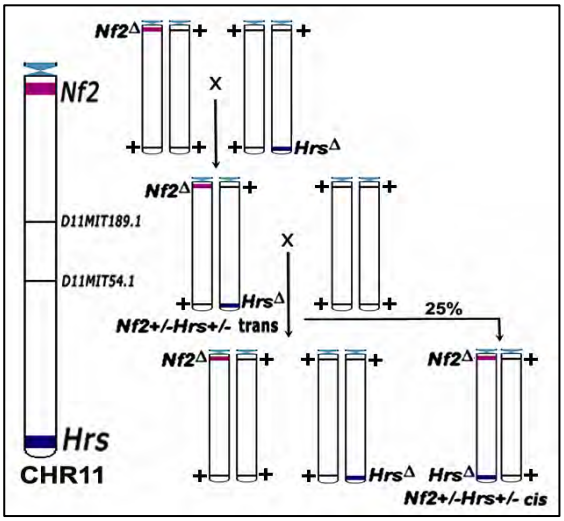
In general and in contrast to previous reports, we were not able to replicate the highly metastatic phenotype of tumors in heterozygous Nf2 mice (McClatchey et al 1998). It is unlikely that these tumors were missed as they usually presented as widely metastasizing osteosarcomas with metastases to lung and liver and usually caused premature death of animals. We neither found evidence of primary tumors nor did we detect any metastases. We need to emphasize the large number of organs that we examined in each animal.

**Non-tumor spectrum**

We also detected abnormalities in our mice that were not tumor related. This involved pancreatic islet cell hyperplasia and presence of cataracts. As tables 1 and 2 show, however, these abnormalities were found in all four genotypes at 18 months. There were no significant differences in the prevalence of cataracts or islet cell hyperplasia. Lenticular abnormalities of the eye were common at 18 months. Cataracts were seen in 41% of animals, but did not show a predilection for a specific genotype.

### *Years 3 & 4: Compound heterozygosity in cis and trans*

We had requested and had received approval to re-focus task 3 of our proposal on the study of the existing various mouse lines and in particular to focus on the analysis of double heterozygotes in *cis* and *trans* with increased numbers and a prolonged time of follow-up. Due to the segregation distortion reported in the prior year of funding, a larger number of animals will be required to generate the requisite genotypes.




The rational for these studies is explained in detail in our original application. In brief (and schematically shown on the left), these studies make use of the observation that *Hrs* and *Nf2* are located on the same chromosome in the mouse. Thus, breeding of mice with the null allele in *cis* (mutations located on the same chromosomal strand) will result in entire loss of Hrs and Nf2 function when proliferating cells stochastically loose the entire mouse chromosome 11.

We have crossed *Nf2*$^{+/-}$;*Hrs*$^{+/-}$*trans* mice with wildtype mice. Reflecting the genetic distance between the two loci we expected to obtain approximately 25% wild-type and 25% compound heterozygotes with the mutant alleles in *cis*. Out of 13 independent matings we had viable litters from 9. Four matings did not produce offspring even when mated with a different mate. In total, 63

pups were produced, but only 5 pups were double heterozygotes. Thus, it appears that there will be significant segregation distortion.

We have transferred breeder pairs and mice of all genotypes to the Biopolymers Vivarium at the University of Utah. We will continue breeding of the required double heterozygotes with wildtype animals to generate animals with the *Nf2*$^{+/-}$;*Hrs*$^{+/-}$ *trans allele.* We estimate that we will breed a much larger number of breeder pairs, because current data suggest that we will obtain double heterozygotes in >10% instead of the expected 25% of pups.

These studies will now be carried out after move of the laboratory to the U. of Utah. We have a sufficient number of breeder pairs to conclude these studies in the remaining time of funding.

## Key Research Accomplishments & Reportable Outcomes

- Intercrosses of *Hrs*$^{+/-}$ mice with *Nf2*$^{+/-}$ (mice *Nf2*$^{+/KO3}$) results in viable offspring with *Nf2*$^{+/-}$ mice born at slightly reduced rates.
- Survival differences could not be detected between the 4 genotypes.
- At 18 months, all 4 lines developed a significant number of microscopic tumors. Prevalence and spectrum of tumors, however, was not significantly different in the four lines.
- The highly metastatic tumor phenotype previously observed could not be replicated in the mixed background. This could point to the importance of as yet unrecognized environmental or genetic background factors in tumor development.
- New mouse lines have been generated that contain Hrs and Nf2 null alleles either in *trans* or in *cis*.

## Conclusions

- Careful necropsy of our various mouse lines has uncovered the presence of microscopic tumors.
- Based on our results, we have focused our studies on mouse lines that carry *Nf2* and *Hrs* null alleles in *cis* and plan for an extended period of observation to capture the full phenotype.
- As recently reviewed (Rivas & Tesarollo, 2008), there is now increasing evidence that mouse background can have a major effect on the phenotypes seen in mice. Our results in a mixed FVB/BL6 background support these conclusions as we have been unable to replicate the highly metastatic phenotype seen in a 129 background. This may point to important genetic modifiers of the tumor phenotype in mice.

## References

McClatchey AI, Saotome I, Mercer K, Crowley D, Gusella JF, Bronson RT, Jacks T. *Genes Dev*. **12(8)**, 1121 (1998).

Rivera J & Tessarollo L. *Immunity*. **28**, 1 (2008).

# Appendices

## Table 1: Tumors in brain and spinal cord and presence of cataracts

| Number | HRS | NF2 | Brain | Spinal Cord | Eye | Meninges |
|---|---|---|---|---|---|---|
| 4 | +/- | +/- | ND | ND | ND | ND |
| 23 | +/- | +/- | N | N | Cataract | N |
| 26 | +/- | +/- | N | N | Cataract | N |
| 27 | +/- | +/- | N | N | Cataract | N |
| 31 | +/- | +/- | N | N | N | Hyperostosis |
| 33 | +/- | +/- | N | N | N | N |
| 38 | +/- | +/- | N | N | N | N |
| 39 | +/- | +/- | N | N | N | N |
| 49 | +/- | +/- | N | N | N | N |
| 51 | +/- | +/- | N | N | N | N |
| 58 | +/- | +/- | Calcification Vasculitis | EIC | N | N |
| 68 | +/- | +/- | N | N | N | N |
| 75 | +/- | +/- | N | N | N | N |
| 77 | +/- | +/- | Calcifications Basal Ganglia | N | N | N |
| 78 | +/- | +/- | N | N | Cataract | N |
| 86 | +/- | +/- | Calcifications Basal Ganglia | N | Cataract | N |
| 87 | +/- | +/- | N | N | Cataract | N |
| 111 | +/- | +/- | N | N | N | N |
| 125 | +/- | +/- | Migration? | N | N | N |
| 132 | +/- | +/- | N | N | N | N |
| F3-57 | +/- | +/- | N | N | Cataract | N |
| F4-17 | +/- | +/- | N | N | N | N |
| F4-20 | +/- | +/- | N | N | N | N |
| F4-23 | +/- | +/- | Calcifications Basal Ganglia | N | Cataract | N |
| F4-24 | +/- | +/- | N | N | Cataract | N |
| F4-25 | +/- | +/- | N | N | N | N |
| F4-26 | +/- | +/- | N | N | N | N |
| F4-28 | +/- | +/- | N | N | Cataract | N |
| HNF2-54 | +/- | +/- | N | N | N | N |
| 35 | +/- | wt | N | N | N | N |
| 41 | +/- | wt | N | N | N | N |
| 47 | +/- | wt | N | N | N | N |
| 48 | +/- | wt | Calcifications Basal Ganglia | N | Cataract | N |
| 50 | +/- | wt | N | N | N | N |
| 55 | +/- | wt | N | N | N | N |
| 56 | +/- | wt | N | N | N | N |
| 57 | +/- | wt | Axonal Degeneration | N | N | N |
| 70 | +/- | wt | N | N | C | N |
| 71 | +/- | wt | Calcifications Basal Ganglia | N | Cataract | N |
| 89 | +/- | wt | N | N | N | N |
| 90 | +/- | wt | N | N | N | N |
| 91 | +/- | wt | N | N | N | N |
| 92 | +/- | wt | Calcifications Basal Ganglia | N | N | N |
| 95 | +/- | wt | N | N | N | N |
| 96 | +/- | wt | Calcifications Basal Ganglia | N | Cataract | N |
| 106 | +/- | wt | N | N | N | N |
| 109 | +/- | wt | N | N | N | N |

| | | | | | | |
|---|---|---|---|---|---|---|
| 110 | +/- | wt | N | N | N | N |
| 119 | +/- | wt | ND | ND | ND | ND |
| 130 | +/- | wt | N | N | N | N |
| F3-100 | +/- | wt | N | N | Cataract | N |
| F3-115 | +/- | wt | Axonal degeneration | Vasculitis | N | Meningitis |
| F3-117 | +/- | wt | N | N | Cataract | N |
| F3-123 | +/- | wt | N | N | N | N |
| F3-98 | +/- | wt | Calcifications Basal Ganglia | N | Cataract | N |
| F4-02M | +/- | wt | N | N | N | N |
| F4-04 | +/- | wt | N | N | N | N |
| F4-31 | +/- | wt | N | N | Cataract | N |
| F4-32 | +/- | wt | N | N | Cataract | N |
| F4-34 | +/- | wt | Calcifications Basal Ganglia | N | Cataract | N |
| F4-02F | +/- | wt | N | N | Cataract | N |
| HNF3-54 | +/- | wt | N | N | N | N |
| | | | | | | |
| 12 | wt | +/- | ND | ND | ND | ND |
| 18 | wt | +/- | N | N | Cataract | N |
| 22 | wt | +/- | N | N | N | N |
| 28 | wt | +/- | N | N | Cataract | N |
| 29 | wt | +/- | Calcifications Basal Ganglia | N | Cataract | N |
| 36 | wt | +/- | N | MPNST | N | Hyperostosis |
| 43 | wt | +/- | N | N | N | N |
| 45 | wt | +/- | N | N | N | N |
| 52 | wt | +/- | N | N | N | N |
| 60 | wt | +/- | N | N | N | N |
| 62 | wt | +/- | Calcifications Basal Ganglia | N | Cataract | N |
| 72 | wt | +/- | N | N | Cataract | N |
| 74 | wt | +/- | N | N | N | N |
| 93 | wt | +/- | N | N | Cataract | N |
| 94 | wt | +/- | N | N | N | N |
| 131 | wt | +/- | N | N | Cataract | N |
| F3-55 | wt | +/- | N | N | N | N |
| F3-59 | wt | +/- | N | N | N | N |
| F3-60 | wt | +/- | N | N | N | N |
| F4-16 | wt | +/- | N | N | Cataract | N |
| F4-19 | wt | +/- | Calcifications Basal Ganglia | N | Cataract | N |
| F4-22 | wt | +/- | Calcifications Basal Ganglia | N | Cataract | N |
| | | | | | | |
| 32 | wt | wt | N | N | N | N |
| 37 | wt | wt | N | N | N | N |
| 42 | wt | wt | N | N | N | N |
| 61 | wt | wt | N | N | N | N |
| 76 | wt | wt | N | N | N | N |
| 88 | wt | wt | N | N | Cataract | N |
| 107 | wt | wt | N | N | N | N |
| 108 | wt | wt | N | N | N | N |
| 113 | wt | wt | N | N | N | N |
| 114 | wt | wt | Agenesis CC Lipoma Calcifications | N | N | N |
| 120 | wt | wt | Calcifications Basal Ganglia | N | ND | N |
| 121 | wt | wt | Calcifications Basal Ganglia | N | Cataract Pigmentary Hyperplasia | N |
| 127 | wt | wt | N | N | N | N |

| | | | | | | |
|---|---|---|---|---|---|---|
| 133 | wt | wt | Calcifications Basal Ganglia | N | Cataract | N |
| 135 | wt | wt | N | N | N | N |
| 157 | wt | wt | N | N | N | N |
| F3-116 | wt | wt | N | N | Cataract | N |
| F3-118 | wt | wt | N | N | Cataract | Meningitis |
| F3-127 | wt | wt | Calcifications Basal Ganglia | N | Cataract | N |
| F3-97 | wt | wt | Calcifications Basal Ganglia | N | Cataract | N |
| F3-99 | wt | wt | N | N | Cataract | N |
| F4-01 | wt | wt | N | N | N | N |
| F4-03 | wt | wt | N | N | N | N |
| F4-05 | wt | wt | N | N | N | N |
| F4-06 | wt | wt | N | N | N | N |
| F4-07 | wt | wt | N | N | Cataract | N |
| F4-08 | wt | wt | N | N | Cataract | N |
| F4-09 | wt | wt | N | N | Cataract | N |
| F4-10 | wt | wt | N | N | Cataract | N |
| F4-11 | wt | wt | N | N | Cataract | N |
| F4-12 | wt | wt | N | N | Cataract | N |
| F4-13 | wt | wt | N | N | N | N |
| F4-14 | wt | wt | N | N | Cataract | N |
| F4-15 | wt | wt | N | N | Cataract | N |
| F4-21 | wt | wt | N | N | Cataract | N |
| F4-33 | wt | wt | Calcifications Basal Ganglia | N | Cataract | N |
| F4-35 | wt | wt | N | N | Cataract | N |
| F4-27 | wt | wt | N | N | Cataract | N |
| F4-29 | wt | wt | N | N | N | N |
| F4-30 | wt | wt | N | N | N | N |
| F4-66 | wt | wt | N | N | Cataract | N |

N: Normal; ND: Not done, usually to death of animal (prior to 18 months) and tissue decomposition
MPNST: Malignant peripheral nerve sheath tumor

## Table 2: Other Tumors

| Number | HRS | NF2 | Lung | Liver | Kidney | Pancreas |
|---|---|---|---|---|---|---|
| 4 | +/- | +/- | ND | ND | ND | ND |
| 23 | +/- | +/- | Adenocarcinoma | N | N | N |
| 26 | +/- | +/- | Adenocarcinoma | Hepatocellular Carcinoma | N | N |
| 27 | +/- | +/- | N | N | Inflammation | N |
| 31 | +/- | +/- | N | N | N | N |
| 33 | +/- | +/- | N | N | N | N |
| 38 | +/- | +/- | N | Nuclear Hyperploidy | N | N |
| 39 | +/- | +/- | N | N | N | N |
| 49 | +/- | +/- | Adenocarcinoma | N | N | N |
| 51 | +/- | +/- | N | N | Inflammation | N |
| 58 | +/- | +/- | N | N | N | N |
| 68 | +/- | +/- | N | N | N | N |
| 75 | +/- | +/- | Inflam | N | N | N |
| 77 | +/- | +/- | Inflam | Fatty change | Inflammation | N |
| 78 | +/- | +/- | N | Inflam | Inflammation | N |
| 86 | +/- | +/- | N | N | N | N |
| 87 | +/- | +/- | N | N | N | Islets Hyper |
| 111 | +/- | +/- | Inflammation | N | Inflammation | N |
| 125 | +/- | +/- | N | Nuclear Hyperploidy | Inflammation | N |
| 132 | +/- | +/- | N | N | Inflammation | N |
| F3-57 | +/- | +/- | Adenocarcinoma | Nuclear Hyperploidy | N | Islets Hyper |
| F4-17 | +/- | +/- | N | Inflammation | N | Islets Hyper |
| F4-20 | +/- | +/- | N | N | Inflammation | N |
| F4-23 | +/- | +/- | N | N | Hydronephrosis | N |
| F4-24 | +/- | +/- | N | N | Inflammation | N |
| F4-25 | +/- | +/- | Adenocarcinoma | N | Inflammation | N |
| F4-26 | +/- | +/- | N | N | Inflammation | N |
| F4-28 | +/- | +/- | N | N | Inflammation | N |
| HNF2-54 | +/- | +/- | N | N | Inflammation | Islets Hyper |
| 35 | +/- | wt | N | N | N | N |
| 41 | +/- | wt | N | N | Inflammation | N |
| 47 | +/- | wt | N | N | N | N |
| 48 | +/- | wt | N | N | Inflammation | N |
| 50 | +/- | wt | N | N | Inflammation | N |
| 55 | +/- | wt | N | N | N | Islets Hyper |
| 56 | +/- | wt | Adenocarcinoma | N | Inflam/Hydronephrosis | N |
| 57 | +/- | wt | N | N | N | N |
| 70 | +/- | wt | N | Inflam | Inflammation | N |
| 71 | +/- | wt | N | N | N | Islet Hyperplasia |
| 89 | +/- | wt | Adenocarcinoma | N | N | Islets Hyper |
| 90 | +/- | wt | N | N | N | N |
| 91 | +/- | wt | N | Fatty change | Inflammation | N |
| 92 | +/- | wt | N | N | N | N |
| 95 | +/- | wt | N | Hyperploidy | Inflammation | N |
| 96 | +/- | wt | N | Fatty | N | N |
| 106 | +/- | wt | N | N | N | N |
| 109 | +/- | wt | N | N | Inflammation | N |
| 110 | +/- | wt | N | N | Inflammation | N |
| 119 | +/- | wt | ARDS Leukemia | Leukemia | Inflammation | ND |
| 130 | +/- | wt | N | N | Inflammation | N |
| F3-100 | +/- | wt | N | N | N | N |
| F3-115 | +/- | wt | N | N | Inflammation | N |
| F3-117 | +/- | wt | N | N | Inflammation | N |
| F3-123 | +/- | wt | N | N | N | N |

| | | | | | | |
|---|---|---|---|---|---|---|
| F3-98 | +/- | wt | N | N | N | N |
| F4-02M | +/- | wt | Adenocarcinoma | Fatty change | Inflammation | N |
| F4-04 | +/- | wt | N | N | Inflammation | N |
| F4-31 | +/- | wt | N | N | Inflammation | N |
| F4-32 | +/- | wt | N | N | N | N |
| F4-34 | +/- | wt | N | N | Inflammation | N |
| F4-02F | +/- | wt | N | N | N | N |
| HNF3-54 | +/- | wt | N | N | Inflammation | N |
| 12 | wt | +/- | ND | ND | ND | ND |
| 18 | wt | +/- | N | N | Hydronephrosis | Islets Hyper |
| 22 | wt | +/- | N | N | N | N |
| 28 | wt | +/- | N | N | Ossifycation/Amyloidoma | Islets Hyper |
| 29 | wt | +/- | N | N | N | Islets Hyper |
| 36 | wt | +/- | Inflam | N | N | Islets Hyper |
| 43 | wt | +/- | N | N | Inflammation | N |
| 45 | wt | +/- | N | N | Inflammation | N |
| 52 | wt | +/- | N | Nuclear Hyperploidy | N | N |
| 60 | wt | +/- | N | Nuclear Hyperdiploidy | N | N |
| 62 | wt | +/- | Adenocarcinoma | Hyperploidy | Inflam/Hydronephrosis | N |
| 72 | wt | +/- | N | Nuclear Hyperdiploidy | Cysts | Islets Hyper |
| 74 | wt | +/- | N | N | Inflammation | N |
| 93 | wt | +/- | N | N | Inflammation | N |
| 94 | wt | +/- | Adenoma/Carcinoma? | Hyperploidy | Inflammation | N |
| 131 | wt | +/- | N | N | N | N |
| F3-55 | wt | +/- | N | Inflammation | N | N |
| F3-59 | wt | +/- | N | Granuloma | N | N |
| F3-60 | wt | +/- | N | N | N | N |
| F4-16 | wt | +/- | Adenocarcinoma | N | N | N |
| F4-19 | wt | +/- | N | Hepatocellular Carcinoma | Hydronephrosis | Islets Hyper |
| F4-22 | wt | +/- | N | N | Inflammation | N |
| 32 | wt | wt | N | N | N | N |
| 37 | wt | wt | N | N | N | N |
| 42 | wt | wt | N | N | N | N |
| 61 | wt | wt | N | N | N | N |
| 76 | wt | wt | N | Granulomas | Inflammation | N |
| 88 | wt | wt | N | Fatty change | N | N |
| 107 | wt | wt | N | N | Inflammation | N |
| 108 | wt | wt | N | N | Inflammation | N |
| 113 | wt | wt | N | N | ND | N |
| 114 | wt | wt | N | N | Inflammation | N |
| 120 | wt | wt | N | N | N | N |
| 121 | wt | wt | N | Fatty | Inflammation | N |
| 127 | wt | wt | N | N | N | N |
| 133 | wt | wt | N | N | N | N |
| 135 | wt | wt | N | N | Inflammation | N |
| 157 | wt | wt | Lobar Pneumonia | Infarct | Acute Pyelonephritis | N |
| F3-116 | wt | wt | N | N | Inflammation | N |
| F3-118 | wt | wt | N | N | Inflammation | N |
| F3-127 | wt | wt | N | N | Hydronephrosis | N |
| F3-97 | wt | wt | N | N | N | N |
| F3-99 | wt | wt | Adenocarcinoma | N | N | N |
| F4-01 | wt | wt | N | N | Inflammation | N |
| F4-03 | wt | wt | N | N | Inflammation | N |
| F4-05 | wt | wt | Adenocarcinoma | N | N | N |
| F4-06 | wt | wt | N | N | Inflammation | N |
| F4-07 | wt | wt | N | N | Inflammation | N |
| F4-08 | wt | wt | N | N | Inflammation | N |

| | | | | | | |
|---|---|---|---|---|---|---|
| F4-09 | wt | wt | N | N | Inflammation | N |
| F4-10 | wt | wt | Adenocarcinoma | N | Inflammation | N |
| F4-11 | wt | wt | N | N | Inflammation | N |
| F4-12 | wt | wt | N | N | Inflammation | N |
| F4-13 | wt | wt | N | N | Segmental disgenesis | N |
| F4-14 | wt | wt | N | N | N | N |
| F4-15 | wt | wt | N | N | N | N |
| F4-21 | wt | wt | N | N | Inflammation | N |
| F4-33 | wt | wt | Adenocarcinoma | Nuclear Hyperploidy | N | N |
| F4-35 | wt | wt | N | N | Inflammation | N |
| F4-27 | wt | wt | N | N | N | N |
| F4-29 | wt | wt | N | N | Inflammation | N |
| F4-30 | wt | wt | N | N | N | N |
| F4-66 | wt | wt | Inflammation | Inflammation | Hydronephrosis/Inflammation | N |

N: Normal; ND: Not done, usually to death of animal (prior to 18 months) and tissue decomposition
MPNST: Malignant peripheral nerve sheath tumor